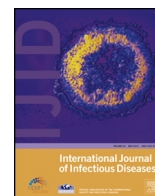


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Short Communication

Detection of the newly characterized HIV CRF56_cpx in Marseille, southeastern France

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SUMMARY

Objectives: We aimed to seek HIV sequences highly similar to CRF56-cpx, a recently described newly circulating B/CRF02/G recombinant HIV, in our local clinical microbiology laboratory sequence database. **Methods:** A recently implemented tool that combines a databank of all HIV nucleotide sequences obtained at our clinical microbiology laboratory with a search tool that uses BLAST was used. A comparative and phylogenetic analysis of HIV protease and reverse transcriptase fragments was performed.

Results: We identified two sequences that were clustered with CRF56-cpx with a bootstrap value of 99% in phylogenetic analyses; these were obtained from two patients diagnosed with HIV in 2009–2011. HIV protease–reverse transcriptase sequences obtained from these two patients shared a mean identity of $98.2 \pm 0.2\%$ with previously described CRF56-cpx sequences. Both case patients diagnosed with HIV in our centre were highly sexually active men who have sex with men.

Conclusions: Our findings highlight the continuous expansion of HIV diversity in France and indicate that real-time surveillance of HIV molecular epidemiology, including the comparison of sequences from laboratory, national, and international databases, might be helpful to identify the emergence, circulation, and transmission of viral strains.

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1. Introduction

A considerable increase in the number of primary HIV infections and other sexually transmitted infections (STIs) has been reported in developed Western countries in recent years.^{1–3} Regarding HIV, transmission clusters involving men who have sex with men (MSM) have been described.^{4,5} In addition, HIV diversity, non-B subtypes, and circulating recombinant forms (CRFs) have been found to be on the rise among MSM in France.^{4,6,7} In the university hospitals of Marseille in southeastern France, we found a considerable increase in STIs diagnosed in our institution in 2012, including gonorrhoea, syphilis, and primary HIV infections

(PHIs), and, concurrently, a 2.2-fold increase in the annual number of MSM exhibiting HIV seroconversion between the periods 2005–2010 and 2011–2012.³

Leoz et al., in Paris, France, recently described a new circulating B/CRF02/G recombinant HIV they named CRF56_cpx.⁶ This complex and second-generation CRF was found to circulate in France in three young MSM (age 21–32 years) diagnosed with PHI in 2009–2011. In addition, a fourth CRF56_cpx sequence from a patient diagnosed with a chronic HIV infection in March 2011, was detected in GenBank using BLAST.⁸

2. Materials and methods

We recently implemented a database that encompasses all HIV and hepatitis virus nucleotide sequences obtained at our clinical microbiology laboratory combined with a search tool that uses

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BLAST and allows the identification of the sequences from this database that are the most similar to sequences recently recovered in our laboratory or released in the NCBI GenBank sequence database (http://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome).^{9,10}

We searched for HIV sequences highly similar to CRF56-cpx in our sequence database, which contains 14 766 HIV protease and 14 673 HIV reverse transcriptase (RT) sequences obtained from plasma samples collected between 1996 and 2014, as described previously.⁹ These HIV sequences correspond to the entire

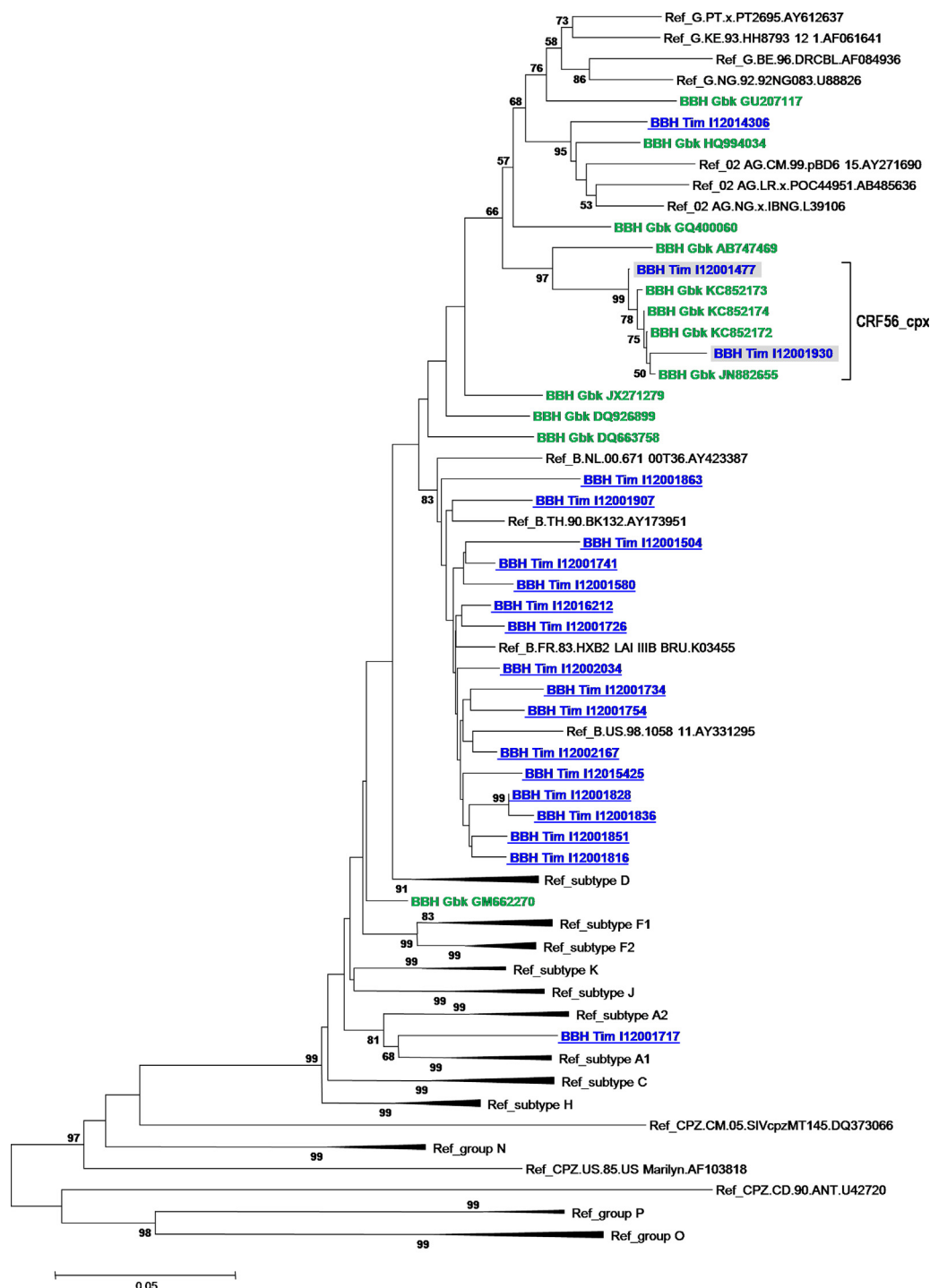


Figure 1. Phylogenetic reconstruction based on an HIV fragment (959 nucleotide positions; nucleotides 1778–2736 in reference to GenBank accession number **L39106**) encompassing the protease gene and the 723 first nucleotides of the reverse transcriptase gene. Sequences analysed were those recovered in the present study, their best BLAST hits in our laboratory,¹⁰ the NCBI nucleotide sequence database (http://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome), and a set of HIV reference genomes from the Los Alamos HIV sequence database (<http://www.hiv.lanl.gov/content/sequence/HIV/mainpage.html>). The sequences described in the present study are highlighted in grey. Sequences corresponding to the best BLAST hits (BBH) for HIV CRF56_cpx and recovered in our laboratory and from the NCBI GenBank nucleotide sequence database are indicated in boldface (in addition, those obtained from our laboratory are underlined). Sequence alignment was performed by the MUSCLE program (<http://www.drive5.com/muscle/>) and the tree was built with MEGA5 software (<http://www.megasoftware.net/>) using the neighbour-joining method. Branches with bootstrap values >50%, obtained from 1000 resamplings of the data, are labelled on the tree. The scale bar indicates the number of nucleotide substitutions per site.

protease gene, the first 723 nucleotides of the RT gene, and the HIV fragment that encompasses these two regions.

3. Results and discussion

We identified two sequences that were clustered with CRF56_cpx in our laboratory database, obtained from two patients in 2012. In addition, in GenBank using BLAST, we detected the fourth CRF56_cpx sequence described by Leoz et al. from a patient diagnosed with chronic HIV infection in March 2011.⁸

Comparative and phylogenetic analyses of the HIV protease–RT, protease, and RT fragments obtained in our laboratory, from Leoz et al.,^{6,8} and from GenBank, showed congruently that sequences obtained from the two patients whose cases are reported here were clustered with CRF56_cpx, with a bootstrap value of 99% (Figure 1; **Supplementary Material**, Figures S1 and S2). HIV protease–RT fragments from these six patients shared a mean identity (\pm standard deviation) of $98.6 \pm 0.8\%$ (range 96.8–99.6%) between each other. Viral sequences from the two patients diagnosed with HIV in Marseille shared a mean identity of $98.2 \pm 0.2\%$ (range 97.9–98.4%) with those described by Leoz et al.^{6,8} As a comparison, the first best hit (GenBank accession number **AB747469**), which branches as an outgroup on the CRF56_cpx cluster and was obtained from a serum sample collected in the Philippines in 2011, and the second best hit detected in GenBank for these six sequences, showed a mean identity of 94.5% and 92.4%, respectively. At the amino acid level, identity between the RT sequences from the present study and those obtained by Leoz et al.,^{6,8} and between these six sequences, ranged from 95.9% to 99.5%.

Both case patients diagnosed with HIV in our centre were highly sexually active MSM. Case 1 was a 22-year-old man diagnosed with chronic HIV infection in 2009. He had received antiretroviral treatment since August 2010 (tenofovir/emtricitabine, plus atazanavir then efavirenz). In November 2012, HIV RNA was undetectable (HIV pol sequences were obtained from peripheral blood mononuclear cells) and his CD4 cell count was 853/mm³. Case 2 was a 31-year-old man diagnosed with chronic HIV infection in 2011. He had received no antiretroviral therapy. In September 2012, his HIV RNA load was 4.1 log₁₀ copies/ml and CD4 cell count was 424/mm³. The four MSM whose HIV sequences were reported by Leoz et al.⁶ originated from France (West French Indies in one case) and were diagnosed with HIV in Paris, although one lives in southeastern France. Based on age, no patient is common to the study of Leoz et al.⁶ and our study.

Taken together with previous findings, our results highlight the continuous expansion of HIV diversity in France, and indicate that this expansion may be boosted by increased HIV transmission among MSM. Real-time surveillance of HIV molecular epidemiology including comparisons of sequences from laboratory, national, and international databases might be helpful to identify the emergence, circulation, and transmission of viral strains.

Conflict of interest: No potential conflict of interest or financial disclosure for all authors.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ijid.2014.10.020>.

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